ChemBioChem

Supporting Information

Hybridization Networks of mRNA and Branched RNA Hybrids

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Contents

1. General

Reagents and solvents were obtained from Merck (Darmstadt) and Carl Roth (Karlsruhe. RNA phosphoramidites (5'-*O*-DMT-2'-*O*-TBDMS-rA^{Ac}, 5'-*O*-DMT-2'-*O*-TBDMS-rU, 5'-*O*-DMT-2'-*O*-TBDMS-rC^{Ac}, 5'-*O*-DMT-2'-*O*-TBDMS-rG^{Ac}) were purchased from FUTUREsynthesis (Poznan, Poland). Oligoribonucleotide strands were obtained from Biomers (Ulm) in HPLC-purified form and were used without further purification.

Chromatography

RP-HPLC chromatography of mRNA biomaterial was performed on a DNAPac™ RP column (2.1x100 mm, 4µm, von *Thermo Fisher Scientific*), using a gradient of MeCN in triethylammonium acetate buffer (0.1 M, pH 7) with a flow rate 0.4 mL/min and a column temperature of 55°C. IEX chromatography of RNA hybrids was performed on a NUCLEOGEL SAX 50/7.7 (semiprep. Column by *Macherey-Nagel*) and Proswift™ SAX-1S 50/4.6 (analytical column by Thermo Scientific) with a salt gradient of sodium perchlorate (0-0.5 M) in Tris buffer (20 mM, pH 7.1). IEX- cartridges Sep-Pak Vac QMA 3cc (1g) were obtained from Waters (Milford, USA) and used with a mixture of Tris- HCl buffer (20 mM, pH 7.1) and sodium perchlorate (0-0.5 M) to purify RNA hybrids. The fractions were analysed by UV at 260 nm and MALDI- TOF mass spectra in linear negative mode.

MALDI-TOF mass spectrometry

RNA hybrid fractions were analyzed in linear negative mode with 2,4-6-trihydroxyacetophenon (0.3 M in EtOH) and diammonium citrate (0.1 M in H_2O) as matrix/comatrix in a ratio of 2:1 (v:v) on a *microFlex* mass spectrometer (Bruker Daltonics). The spectra were analyzed with *flexAnalysis 3.4*.

UV measurements

UV melting curves were performed in Tris-HCl buffer (10 mM, 1 M NaCl, pH 7.1) and measured at 260 nm on a *Lambda-25* spectrometer (Perkin Elmer) with *TempLab 2.0* software. UV analysis of oligonucleotide fractions were analyzed by *NanoDrop 2000c* spectrometer (*d*= 0.1 cm) (Thermo Fisher).

NMR spectroscopy

The NMR spectra were measured on a Bruker Avance III HD spectrometer (700 MHz, 5 mm helium-cooled QCI CryoProbe). Bruker Match™ tubes (3 mm) and Deuterium oxide (D_2O; 99.9 % deuterated) from Euriso-Top (Saint-Aubin, France) were used. ^{1}H-NMR spectra (Bruker pulse sequence noesygppr1d) were recorded with presaturation during relaxation delay (d1 = 2 s) and a mixing time of 10 ms, using. The ^{1}H-NMR spectra were recorded with 16 scans and 64k data points (zero-filled to 128k data points). ^{13}C- and ^{31}P-NMR spectra were acquired with broadband proton decoupling and a 30 degree flip angle (zgpg30). The following pulse sequences from the Bruker library were used: cosygpprqf (^{1}H COSY) mlevphpr.2 (^{1}H TOCSY), hsqcedetgpsisp2.3 (^{1}H-^{13}C HSQC) and the sequence hmbcgplpndprqf for ^{1}H-^{31}P HMBC. The HMBC and HSQC spectra were acquired in the non-uniform sampling mode with 25% NUS-amount. The Bruker Software TopSpin 4.0.9 was used to process and analyze the spectra.

Full mRNA sequence

The mRNA was kindly provided by CureVac (Tübingen) and is 1957 nucleotides in length. The full sequence of the mRNA is:[S1]

GGGGCGCUGCCUACGGAGGUGGCAGCCAUCUCCUUCUCGGCAUCAAGCUUACC
AUGGUGCCCCAGGCCCUGCUCUUCGUCCCGCUGCUGGUGUUCCCCCUCUGCUUC
GGCAAGUUCCCCAUCUACACCAUCCCCGACAAGCUGGGGCCGUGGAGCCCCAUC
GACAUCCACCACCUGUCCUGCCCCAACAACCUCGUGGUCGAGGACGAGGGCUG
CACCAACCUGAGCGGGUUCUCCUACAUGGAGCUGAAGGUGGGCUACAUCAGCG
CCAUCAAGAUGAACGGGUUCACGUGCACCGGCGUGGUCACCGAGGCGGAGACC
UACACGAACUUCGUGGGCUACGUGACCACCACCUUCAAGCGGAAGCACUUCCG
CCCCACGCCGGACGCCUGCCGGGCCGCCUACAACUGGAAGAUGGCCGGGGACCC
CCGCUACGAGGAGUCCCUCCACAACCCCUACCCCGACUACCACUGGCUGCGGAC
CGUCAAGACCACCAAGGAGAGCCUGGUGAUCAUCUCCCCGAGCGUGGCGGACC
UCGACCCCUACGACCGCUCCCUGCACAGCCGGGUCUUCCCCGGCGGGAACUGCU
CCGGCGUGGCCGUGAGCUCCACGUACUGCAGCACCAACCACGACUACACCAUCU
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GGGCCUCUACAAGUCCCUGAAGGGGGCCUGCAAGCUGAAGCUCUGCGGCGUGC
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ACCAAGUGGUGCCCCCCCGGCCAGCUGGUCAACCUGCACGACUUCCGGAGCGAC
GAGAUCGAGCACCUCGUGGUGGAGGAGCUGGUCAAGAAGCGCGAGGAGUGCCU
GGACGCCCUCGAGUCCAUCAUGACGACCAAGAGCGUGUCCUUCCGGCGCCUGA
GCCACCUGCGGAAGCUCGUGCCCGGGUUCGGCAAGGCCUACACCAUCUUCAAC
AAGACCCUGAUGGAGGCCGACGCCCACUACAAGUCCGUCCGCACGUGGAACGA
GAUCAUCCCGAGCAAGGGGUGCCUGCGGGUGGGCGGCCGCUGCCACCCCCACG
UCAACGGGGUGUUCUUCAACGGCAUCAUCCUCGGGCCCGACGGCAACGUGCUG
AUCCCCGAGAUGCAGUCCAGCCUGCUCCAGCAGCACAUGGAGCUGCUGGUCUC
CAGCGUGAUCCCGCUCAUGCACCCCCUGGCGGACCCCUCCACCGUGUUCAAGAA
CGGGGACGAGGCCGAGGACUUCGUCGAGGUGCACCUGCCCGACGUGCACGAGC
GGAUCAGCGGCGUCGACCUCGGCCUGCCGAACUGGGGGAAGUACGUGCUGCUC
UCCGCCGGCGCCCUGACCGCCCUGAUGCUGAUCAUCUUCCUCAUGACCUGCUGG
CGCCGGGUGAACCGGAGCGAGCCCACGCAGCACAACCUGCGCGGGACCGGCCG
GGAGGUCUCCGUGACCCCGCAGAGCGGGAAGAUCAUCUCCAGCUGGGAGUCCU
ACAAGAGCGGCGGCGAGACCGGGCUGUGAGGACUAGUGCAUCACAUUUAAAAG
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UCAUCUCUUUUUCUUUUUCGUUGGUGUAAAGCCAACACCCUGUCUAAAAAACA
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GGAAAGAACCUAGAUCUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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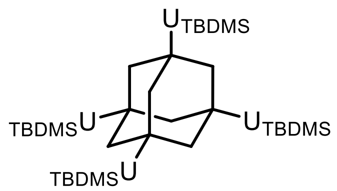
CCCCCCCCCCCCAAAGGCUCUUUUCAGAGCCACCAGAAUU

2. Synthesis of RNA hybrids

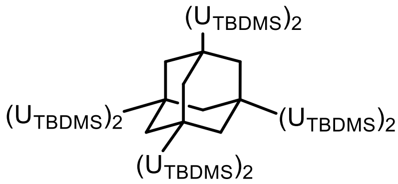
Protected $(U)_4$TOA (2)- (General Protocol A)



This protocol for the synthesis of (5'-OH-2'-TBDMS-$U_{(CE)})_4$TOA (**2**) from tetraol **1** and is representative. Conceptually, it is a modification of a protocol for the solution-phase synthesis of DNA hybrids.[S2] First 1,3,5,7-adamantane tetraol (**1**, 20 mg, 0.1 mmol, 1 equiv.), the commercially available uridine phosphoramidite building block (688 mg, 0.8 mmol, 8 equiv) and molecular sieves (3Å) were dried in vacuo ($<10^{-3}$ mbar) for 3 h. Under argon atmosphere, anhydrous DMF (1.85 mL) and 1*H*-tetrazole solution (2.66 mL, 0.45 M in MeCN, 12 equiv.) were added. The components were fully dissolved using an ultrasonic bath, and the flask was shaken first for 30 min at room temperature and finally kept at 4 °C for 24 hours. After this, *tert*-butyl hydroperoxide solution (540 µL, 1.2 mmol, 5.5 M in *n*-decane, 12 equiv) was added, and the reaction mixture was kept at 4 °C for 20 min. Then, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with phosphate buffer (30 mL, 0.2 M, pH 7). The organic phase was separated, and the aqueous phase was back-extracted with CH_2Cl_2 (3× 40 mL). The organic layers were combined and dried with Na_2SO_4, filtered and concentrated *in* vacuo. The residue was coevaporated twice from MeOH and then dissolved in a minimal amount of CH_2Cl_2 (2 mL), followed by precipitation with cold diisopropyl ether (10 mL) and separation by centrifugation (3000 g, 2 min). This process was repeated twice. Detritylation was performed by dissolving the residue in CH_2Cl_2 (5 mL), addition of water (50 µL) and dichloroacetic acid (7.5 mL, 6 % in CH_2Cl_2). After 10 min, the reaction was stopped by addition of methanol (4 mL), the volume was reduced to 2 mL, and the RNA hybrid was precipitated with cold diisopropyl ether (5 mL). After separation by centrifugation (3000 g, 2 min), the residue was dissolved in a mixture of CH_2Cl_2 and methanol (1 mL, 5/1, *v/v*) and precipitated again with cold diisopropyl ether (5 mL). This process was repeated twice. After drying, RNA hybrid **2** was obtained as a colourless solid (377 mg, 1.94 mmol, 97 % crude). For the next coupling step, RNA hybrid **2** was used without further purification. For mass spectrometric analysis, basic deprotection with AMA

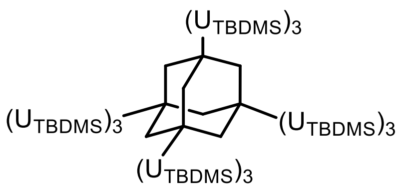
(25 % NH_4OH / 40 % methylamine in H_2O, 1:1) was carried out. MALDI-TOF-MS (without cyanoethyl groups): *m/z* calcd. for $C_{70}H_{116}N_8O_{36}P_4Si_4$ $[M-H]^-$ 1881, obsd. 1880.

Protected (UU)$_4$TOA (**7**).



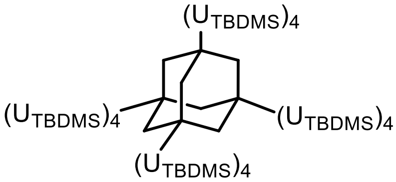
The synthesis of hybrid **7** followed General Protocol A. For this RNA hybrid **2** (205 mg, 98 μmol, 1 equiv.), uridine phosphoramidite (675 mg, 0.8 mnol, 8 equiv.) and tetrazole solution (0.45 M, 2.6 mL, 1.2 mmol, 12 equiv.) in anhydrous DMF (2.2 mL) were stored for 42 h at 4 °C. After oxidation with *t*BuOOH solution (218 μL, 1.2 mmol, 12 equiv.), detritylation with CH_2Cl_2 (5 mL), water (50 μL), and DCA solution (8 mL, 6% in CH_2Cl_2) was performed and crude hybrid was precipitated twice from CH_2Cl_2/MeOH (2 mL, 5/1, *v/v*) with cold diisopropyl ether (5mL). The desired RNA hybrid **7** was isolated in a yield of 377 mg (94 μmol, 96 %, crude). The product was used in the next coupling step without further purification, for mass analysis basic deprotection with AMA was carried out as described above. MALDI-ToF-MS (without cyanoethyl groups): *m/z* calcd. for $C_{129}H_{213}N_{16}O_{68}P_8Si_8$ $[M-H]^-$ 3562, obsd 3565.

Protected (UUU)$_4$TOA (**8**).



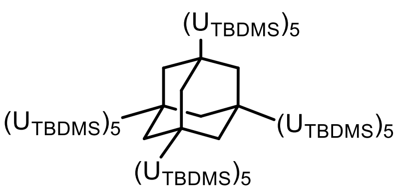
The synthesis was carried out analogous to General Protocol A, starting from hybrid **7** (100 mg, 25 μmol, 1 equiv.), uridine phosphoramidite (173 mg, 201 μmol, 8 equiv.), tetrazole solution (0.45 M, 668 μL, 12 equiv.) in anhydrous DMF (513 μL) for 42 h at 4 °C. The oxidation was carried out with *t*BuOOH solution (5.5 M, 55 μL), followed by detritylation with CH_2Cl_2 (3 mL), DCA solution (4 mL, 6 % in CH_2Cl_2) and water (20 μL). The crude product was precipitated twice from CH_2Cl_2/MeOH (1 mL, 5/1, *v/v*) with cold diisopropyl ether (5 mL). RNA hybrid **8** was obtained in a yield of 125 mg (21 μmol, 88 %, crude). The crude product was used in the next coupling step without further purification, for mass analysis basic deprotection was carried out as described above. MALDI-ToF-MS (without cyanoethyl groups): *m/z* calcd. for $C_{190}H_{315}N_{24}O_{100}P_{12}Si_{12}$ $[M-H]^-$ 5244, obsd 5246.

Protected (UUUU)$_4$TOA (9).



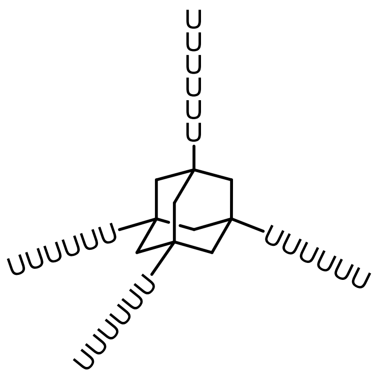
General Protocol A was followed to synthesize RNA hybrid **9**, starting from RNA hybrid **8** (125 mg, 21 μmol, 1 equiv.), uridine phosphoramidite (146 mg, 170 μmol 8 equiv.), tetrazole solution (0.45 M, 490 μL, 12 equiv.) in DMF (377 μL). After storage for 24 h at 4 °C, oxidation with *t*BuOOH solution (40 μL) and then detritylation with CH_2Cl_2 (4 mL), water (20 μL) and DCA solution (6 % in CH_2Cl_2, 6 mL) was carried out. The RNA hybrid **9** was precipitated twice from CH_2Cl_2/MeOH (1 mL, 5/1, *v/v*) with cold diisopropylether (4 mL) and isolated in yield of 160 mg (20 μmol, 95 %, crude). For mass spectrometric analysis, deprotection with base was carried out as described above. The product was used in the next coupling step without further purification. MALDI-ToF-MS (without cyanoethyl groups): *m/z* calcd. for $C_{250}H_{415}N_{32}O_{132}P_{16}Si_{16}$ $[M-H]^-$ 6919, obsd. 6916.

Protected (UUUUU)$_4$TOA (10).



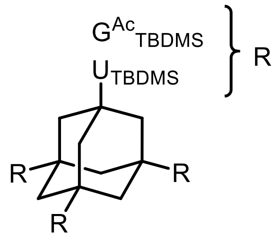
RNA hybrid **10** was prepared following General Protocol A. First hybrid **9** (251 mg, 32 μmol, 1 equiv.), uridine phosphoramidite (277 mg, 320 μmol, 10 equiv.) and tetrazole solution (860 μL, 387 μmol, 12 equiv.) in anhydrous DMF (661 μL) were mixed and stored for 42 h at 4°C. After this *t*BuOOH (5.5 M, 71 µL, 387 µmol, 12 equiv.) was added and work up was performed. DMT groups were removed with CH_2Cl_2 (5 mL), water (30 µL) and DCA solution (6.5 mL, 6 % in CH_2Cl_2) and precipitated from CH_2Cl_2/MeOH (1 mL, 5/1, *v/v*) with cold diisopropyl ether (5 mL) yielded 254 mg (26 µmol, 81 %) of crude RNA hybrid **10**. The crude product was used in the next coupling step without further purification, for mass spectrometric analysis, deprotection with base was carried out as described above. MALDI-ToF-MS (without cyanoethyl groups): *m/z* calcd. for $C_{310}H_{515}N_{40}O_{164}P_{20}Si_{20}$ $[M\text{-}H]^-$ 8608, obsd .8614.

(UUUUUU)$_4$TOA (4).



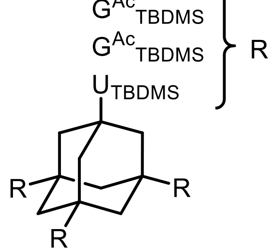
The synthesis of hybrid **4** followed General Protocol A. For this hybrid **10** (254 mg, 26 µmol, 1 equiv.), uridine phosphoramidite (182 mg, 208 µmol, 8 equiv.) and tetrazole solution (0.45 M, 704 µL, 312 µmol, 12 equiv.) in anhydrous DMF (542 µL) were stored for 48 h at 4 °C. Oxidation with *t*BuOOH solution (60 µL, 312 µmol, 12 equiv.), detritylation with CH_2Cl_2 (3 mL), water (20 µL), and DCA solution (4 mL, 6 % in CH_2Cl_2) was performed and crude hybrid was precipitated twice from CH_2Cl_2/MeOH (2 mL, 5/1, *v/v*) with cold diisopropyl ether (5mL). Basic deprotection was carried out with ammonia/ethanol (3 mL, 25 % $NH_4OH_{(aq)}$/ ethanol, 3/1, *v/v*) for 2 h at room temperature. Excess of ammonia was removed by a gentle stream of N_2 stream onto the solution. The lyophilized crude hybrid was desilylated with triethylammonium hydrofluoride (1 mL) for 7 h at room temperature, following by quenching with methoxytrimethylsilane (7 mL) for 30min at room temperature. Hexamer RNA hybrid **4** was purified by IEX-HPLC with Tris-HCl buffer (pH 7.1) using a $NaClO_4$ gradient (0-500 mM). t_R= 24.3 min. Fully deprotected hexamer RNA hybrid **4** was isolated in a yield of 13.6 mg (1.8 µmol, 14 %). ^{1}H-NMR (700 MHz, D_2O and Tris HCl buffer): δ 2.22 (s, CH_2, adamantane, 12H), 3.74 (dd, J=13 Hz, J= 4 Hz, H5'/H5", 4H), 3.82 (dd, J= 13 Hz, J=3 Hz, H5'/H5", 4 H), 4.01-4.09 (m, H5'/H5", 21 H), 4.10-4.19 (m, H5'/H5", 20 H), 4.20-4.24 (m, H4', 5 H), 4.30-4.43 (m, H2', H4', 44 H), 4.47-4.49 (m, H3', 6 H), 4.54-4.63 (m, H3', 18 H), 5.77(d, H5, 4 H), 5.78-5.83 (m, H5, H1', 24 H) 5.78-5.89 (m, H1', 20 H), 7.75-7.81 (m, H6, 24 H) ppm. ^{31}P-NMR (125 MHz, D_2O): -0.78, -4.39 ppm. MALDI-ToF-MS: m/z calcd. for $C_{226}H_{280}N_{48}O_{196}P_{24}$ $[M-H]^-$ 7548, obsd 7547.

Protected (GU)$_4$TOA (11).



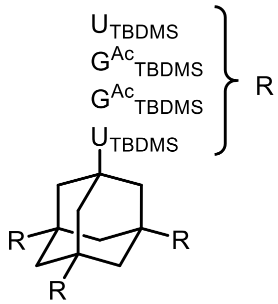
The synthesis was carried out analogous to General Protocol A, starting from hybrid **2** (53 mg, 25 µmol, 1 equiv.), guanosine phosphoramidite (191 mg, 0.2 mmol, 8 equiv.) in anhydrous DMF (0.5 mL) and tetrazole solution (0.68 mL, 0.3 mmol, 12 equiv.) for 24 h in 4 °C. Then *t*BuOOH (56 µL, 0.3 mmol, 12 equiv.) was added. After work up, DMT deprotection with CH_2Cl_2 (3 mL), water (13 µL) and DCA (1.6 mL, 6 % in CH_2Cl_2) was carried out. Finally, the RNA hybrid **11** was precipitated with cold diisopropyl ether (5mL) and dried to obtain 91 mg (21 µmol, 84 %, crude). The product was used in the next coupling step without further purification, for mass spectrometric analysis, basic deprotection with AMA was carried out as described above. **MALDI-TOF-MS** (without cyanoethyl- and acetyl groups): m/z calcd. for $C_{134}H_{219}N_{28}O_{64}P_8Si_8$ $[M-H]^-$ 3718, obsd. 3721.

Protected (GGU)$_4$TOA (**12**).



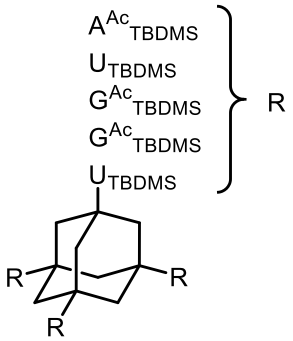
General Protocol A was followed to synthesize RNA hybrid **12**, starting from hybrid **11** (91 mg, 21 μmol, 1 equiv.), guanosine phosphoramidite (238 mg, 252 μmol, 12 equiv.) in DMF (370 μL) and tetrazole solution (0.45 M, 560 μL, 252 μmol, 12 equiv.) for 20 h at 4 °C. After this *t*BuOOH (5.5 M, 50 μL, 252 mmol, 12 equiv.) was added and work up was performed. DMT groups were removed with CH_2Cl_2 (2 mL), water (26 μL) and DCA solution (3.3 mL, 6 % in CH_2Cl_2) and precipitated from CH_2Cl_2/MeOH (1 mL, 5/1, *v/v*) with cold diisopropyl ether (5 mL). The detritylated hybrid **12** was isolated in a yield of 111 mg (17 mmol, 81 %, crude). This product was used in the next coupling step without further purification. For mass spectrometric analysis, basic deprotection of a sample was carried out as described above. **MALDI- TOF-MS** (without cyanoethyl- and acetyl groups): *m/z* calcd. for $C_{198}H_{322}N_{48}O_{92}P_{12}Si_{12}$ $[M-H]^-$ 5555, obsd. 5555.

Protected (UGGU)$_4$TOA (13).



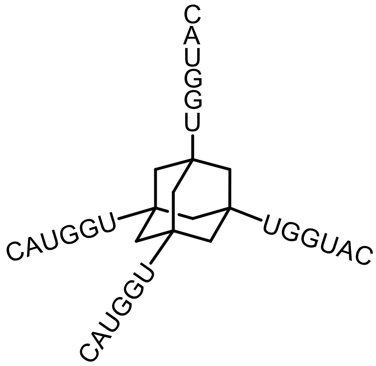
RNA Hybrid **13** was prepared following General Protocol A. First hybrid **12** (111 mg, 17 μmol, 1 equiv.), uridine phosphoramidite (176 mg, 204 mmol, 12 equiv.) in anhydrous DMF (300 μL) and tetrazole solution (0.45 M, 450 μL, 12 equiv.) were mixed together an stored for 29 h at 4 °C. After oxidation with *t*BuOOH solution (5.5 M, 37 μL, 204 μmol, 12 equiv.) and aqueous work up DMT deprotection with CH_2Cl_2 (1 mL), DCA solution (6 % in CH_2Cl_2, 1.3 mL) and water (10 μL) was performed. The solution was concentrated to a total volume of 1 mL and the hybrid was precipitated twice in CH_2Cl_2/MeOH (1 mL, 4/1, *v/v*) with cold diisopropyl ether to obtain RNA hybrid **13** in a yield of 104 mg (12 μmol, 73 %, crude). The product was used in the next coupling step without further purification. For mass spectrometric analysis, deprotection of a sample with AMA was carried out as described above. **MALDI- TOF-MS** (without cyanoethyl- and acetyl groups): *m/z* calcd. for $C_{258}H_{422}N_{56}O_{124}P_{16}Si_{16}$ $[M-H]^-$ 7237, obsd 7243.

Protected (AUGGU)$_4$TOA (14).



General Protocol A was used, starting from hybrid **13** (104 mg, 12 μmol, 1 equiv.) and adenosine phosphoramidite (138 mg, 149 μmol, 12 equiv.) in DMF (280 μL) and tetrazole solution (0.45 M, 330 μL, 149 μmol, 12 equiv.) for 74 h at 4 °C. After oxidation with *t*BuOOH solution (5.5 M, 27 μL, 12 equiv.), DMT removal with CH_2Cl_2 (0.8 mL), water (10 μL) and DCA solution (6 % in CH_2Cl_2, 1.2 mL) and precipitation from CH_2Cl_2/MeOH (1 mL, 4/1, *v/v*) with cold diisopropylether (6 mL). The desired detritylated RNA hybrid **14** was isolated in a yield of 82 mg (9 μmol, 80 %, crude). The crude product was used in the next coupling step without further purification, for mass spectrometric analysis, basic deprotection was carried out as described above. **MALDI- TOF-MS** (without cyanoethyl- and acetyl groups): *m/z* calcd. for $C_{322}H_{527}N_{76}O_{148}P_{20}Si_{20}$ $[M-H]^-$ 9012, obsd 9013.

(CAUGGU)$_4$TOA (6).



RNA hybrid **6** was prepared following General Protocol A. First hybrid **14** (40 mg, 3.8 μmol, 1 equiv.), cytidine phosphoramidite (41 mg, 45 μmol, 12 equiv.) and tetrazole solution (0.45 M, 100 μL, 45 μmol, 12 equiv.) in anhydrous DMF (83 μL) were stored for 74 h at 4 °C. Oxidation with *t*BuOOH solution (5.5 M, 9 μL, 45 μmol, 12 equiv.) was then performed followed by DMT removal with CH_2Cl_2 (0.8 mL), water (10 μL) and DCA solution (1.2 mL, 6 % in CH_2Cl_2). The hybrid was precipitated twice from CH_2Cl_2/MeOH (1 mL) with cold diisopropyl ether. Basic deprotection was carried out with ammonia/methylamine (1.5 mL, 25 % $NH_4OH_{(aq)}$/ 40 %$MeNH_{2(aq)}$, 1/1, *v/v*) at 50 °C for 15 min. Excess of ammonia and methylamine were removed by an N_2 stream directed onto the solution. The lyophilized crude hybrid was desilylated with triethylammonium hydrofluoride (0.7 mL) for 6 h at room temperature, following by quenching with methoxytrimethylsilane (6 mL) for 30 min at room temperature. The crude was the purified on a Sep-Pak IEX cartridge (500 mg) with Tris-HCl buffer (pH 7.1) using a $NaClO_4$ gradient (0-500 mM). Fully deprotected pure RNA hybrid **6** was isolated in a yield of 24 mg (3 μmol, 6 % to 50 μmol TOA). ^{1}H-NMR (700 MHz, 313 K, D_2O and Tris HCl buffer): δ 2.38 (s, CH_2, adamantane, 12 H), 3.86 (dd, *J*=13, *J*= 4 Hz, H5'/H5'', 4 H), 3.95 (dd, H5'/H5'', 4 H), 4.17-4.59 (m, H5'/H5'', H4', H2', H3', 45 H), 4.77-4.88 (m, H2', H2'-A, H2'-G, no proper integration because of water suppression), 5.75 (d, *J*= 8 Hz, H5-U, 4H), 5.78-5.83 (m, H5-U, H1'-G, H1'- U, 16 H), 5.85-5.91 (m, H1'-G, H5-C, 10 H), 5.96-6.02 (m, H1'-U, 4 H), 6.08-6.11 (m, H1'-A, 4 H), 7.74 (d, *J*= 8 Hz, H6-U, 4 H), 7.78 (d, *J*= 7 Hz, H6-C, 4 H), 7.83 (d, *J*= 7 Hz, H6-U, 4 H), 7.95 (s, H2-G, 4 H), 8.01 (s, H2-G, 4 H), 8.19 (s, H2-A, 4 H), 8.41 (s, H8-A, 4 H) ppm. MALDI-ToF-MS: *m/z* calcd. for $C_{238}H_{297}N_{88}O_{177}P_{24}$ $[M-H]^-$ 7965, obsd 7962.

3. NMR spectra

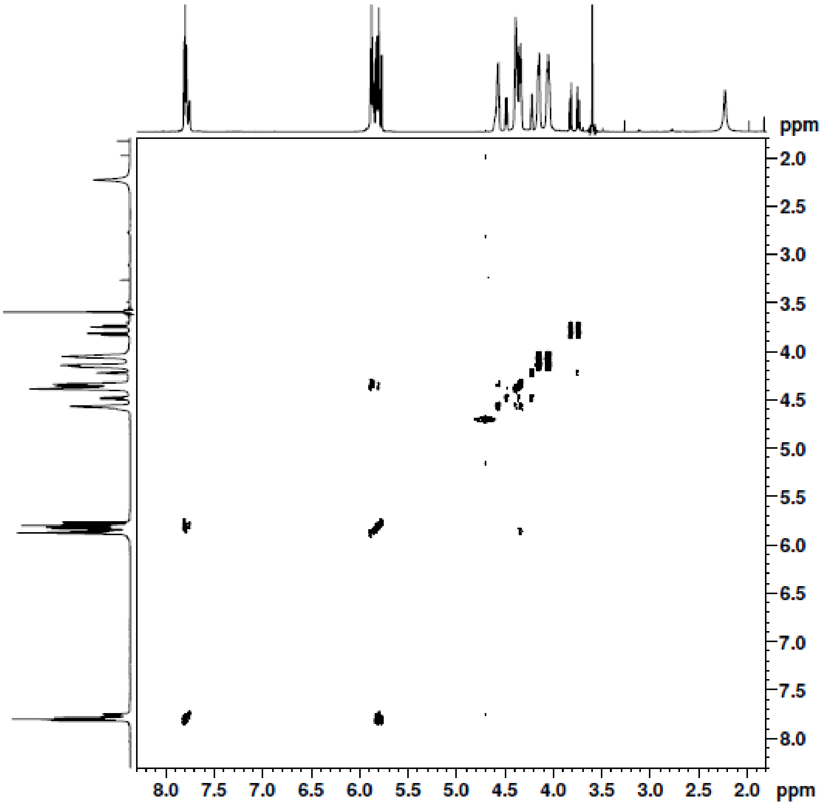


Figure S1. COSY spectrum of (UUUUUU)$_4$TOA (**4**) (D_2O, Tris buffer, 700 MHz).

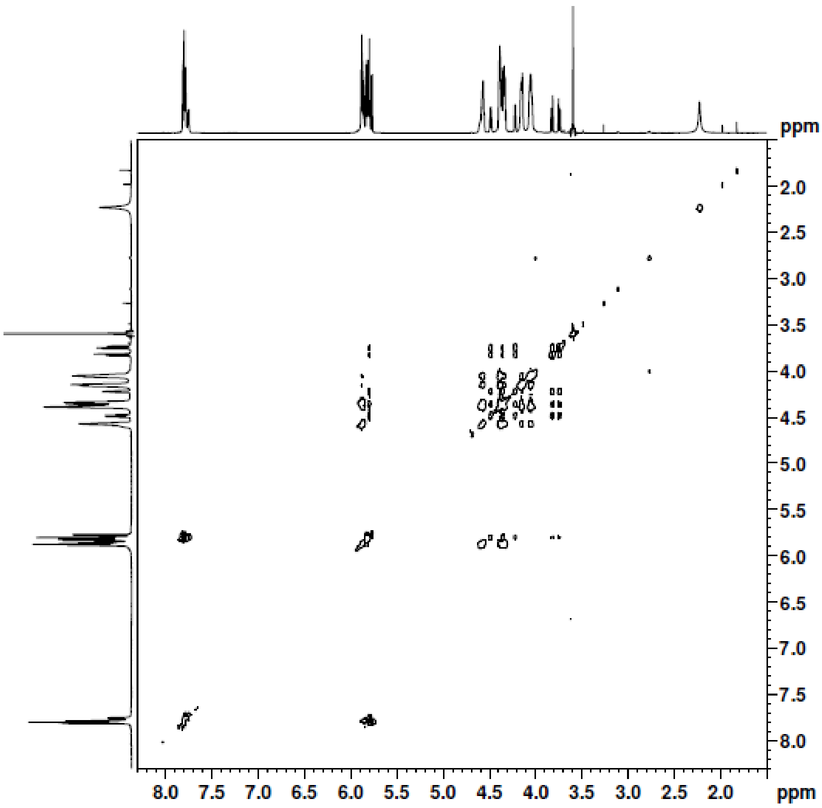


Figure S2. TOCSY spectrum of (UUUUUU)$_4$TOA (**4**) (D_2O, Tris buffer, 700 MHz).

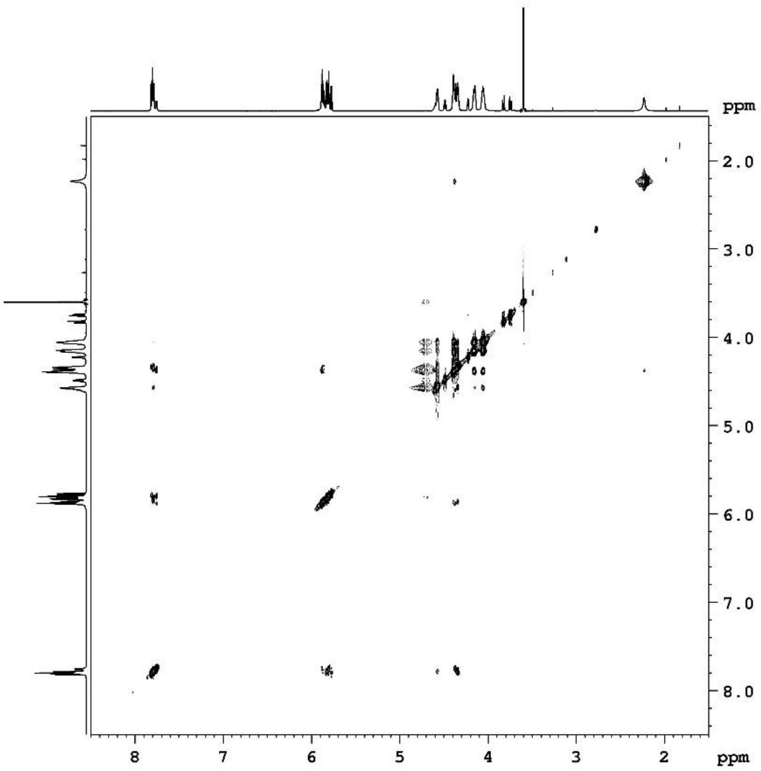


Figure S3. NOESY spectrum of $(UUUUUU)_4$TOA (**4**) (D_2O, Tris buffer, 700 MHz).

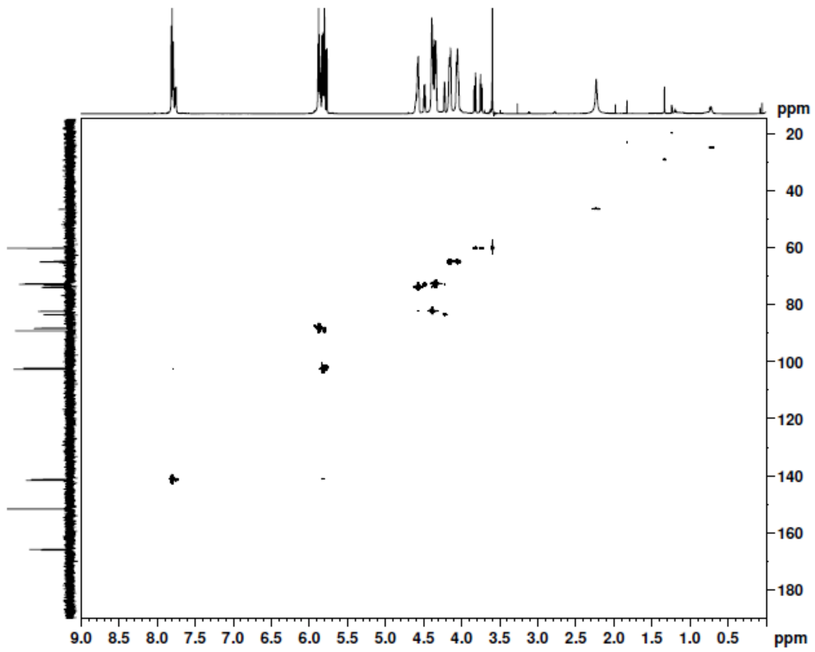


Figure S4. HSQC spectrum of $(UUUUUU)_4$TOA (**4**) (D_2O, Tris buffer, 700 MHz).

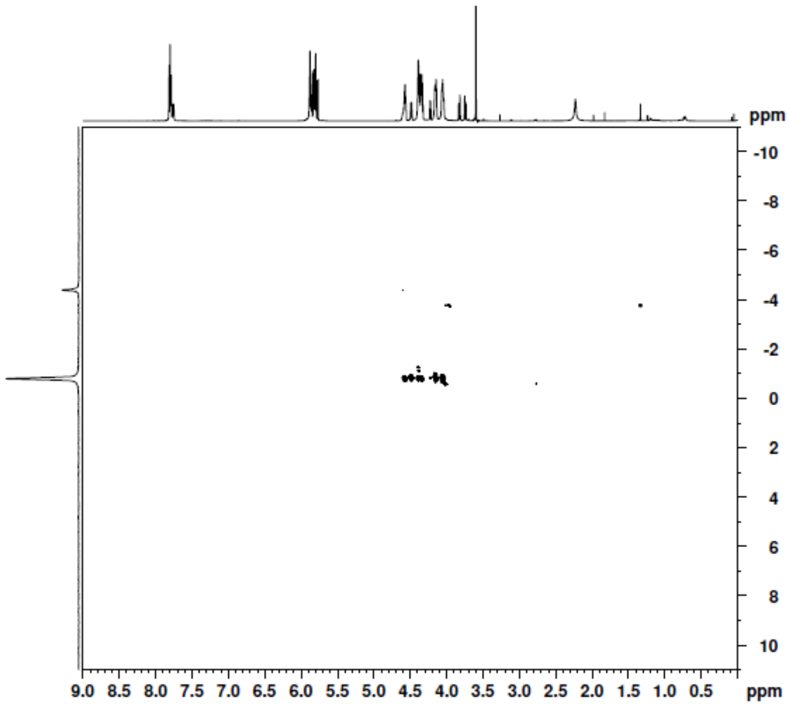


Figure S5. HMBC spectrum of (UUUUUU)$_4$TOA (**4**) (D_2O, Tris buffer, 700 MHz).

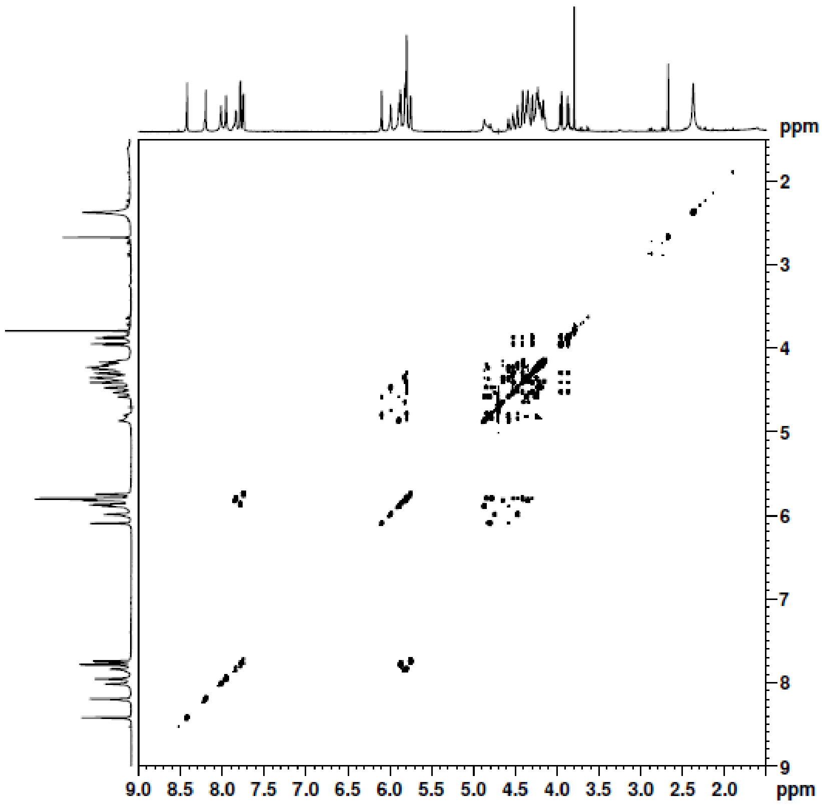


Figure S6. TOCSY spectrum of (CAUGGU)$_4$TOA (**6**) (700 MHz, D_2O, Tris HCl buffer 7.4, 313 K).

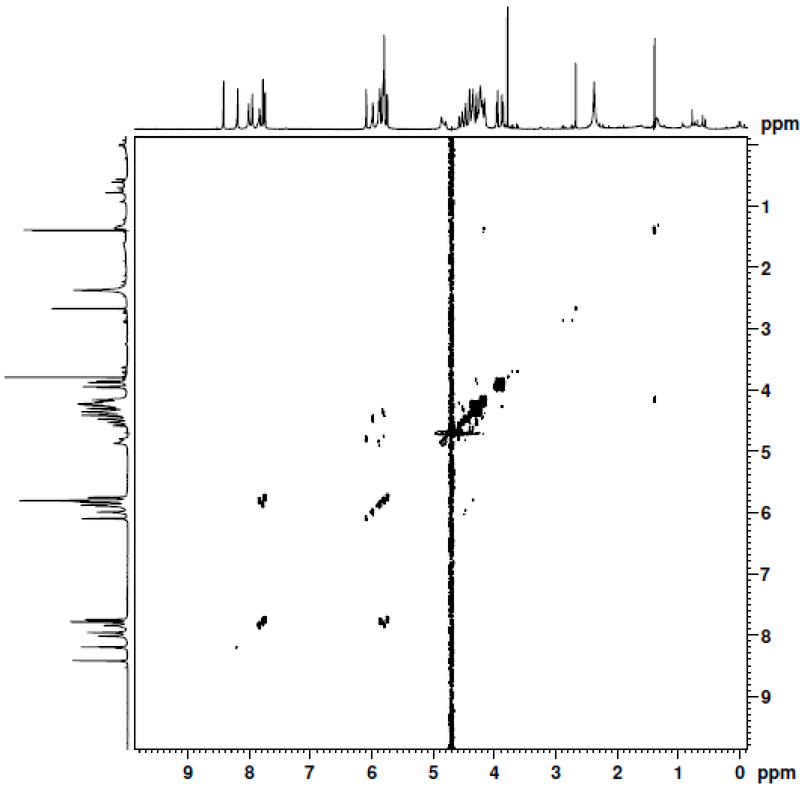


Figure S7. COSY spectrum of (CAUGGU)$_4$TOA (**6**) (700 MHz, D_2O, Tris HCl buffer 7.4, 313 K).

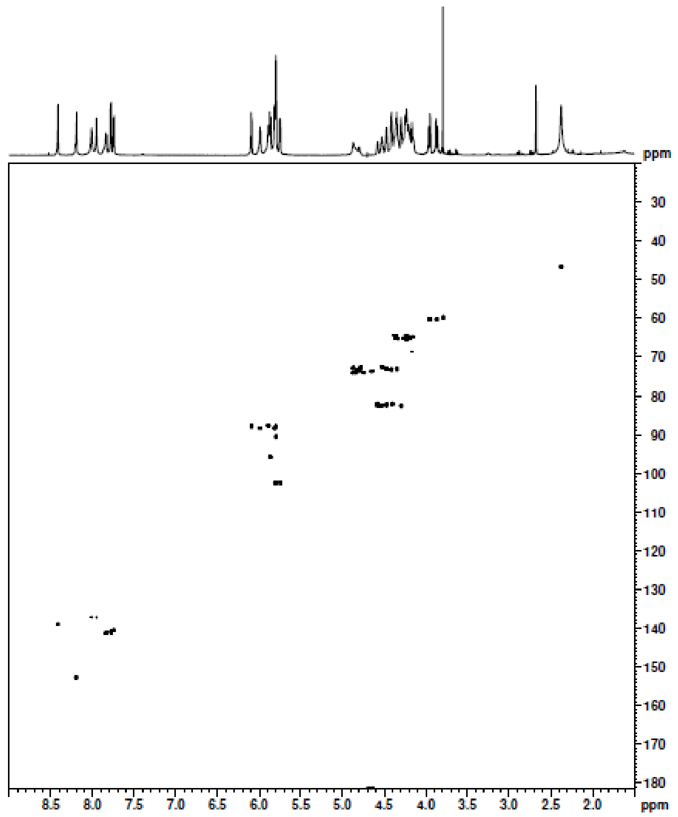


Figure S8. HSQC spectrum of (CAUGGU)$_4$TOA (**6**) (700 MHz, D_2O, Tris HCl buffer 7.4, 313 K).

4 Material-Forming Assays

Biomaterial formation. Assembly experiments and biomaterial formation of mRNA with RNA hybrids **4** and **6** were performed in PCR microtubes. For this, the mRNA (4 μM) was lyophilized and then dissolved in Tris buffer (total volume 30 μL,10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 7.4), followed by the addition of both RNA hybrids at the given ratio (Table S1, experiments 1-7). The mixture was heated to 80 °C for 5 min and cooled to 4 °C in 3 h. The solution was kept at 4 °C for 72 h. The precipitate was analyzed by denaturating PAGE (7 M urea). For this, the supernatant of the sample was removed with a small syringe. The solid was diluted with deionized water (20 μL) and formamide solution (20 μL, 90 % formamide, 1x Tris-EDTA buffer, Orange G), then heated to 80 °C for 5 min and afterwards put on ice for 1 min. Then, the samples and reference samples were placed in the pockets of the gel. The gel was run for 45 min at 200 V and analyzed using UV shadowing (Figure 5).
Additionally, a native agarose gel (1%) in 1x TAE buffer (pH 7) was run to analyze the mRNA biomaterial formed from the mRNA and hybrid **4**. This is shown in Figure S9, below.

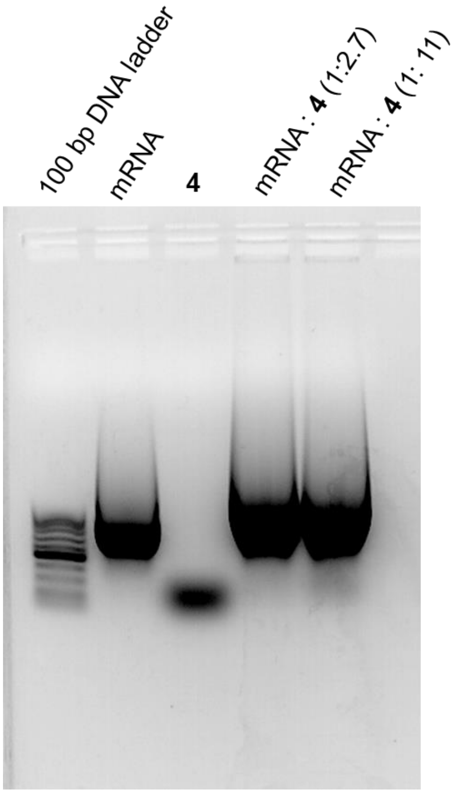


Figure S9. Native agarose gel (1%) of the components of the biomaterial formed of mRNA and RNA hybrid **4** in annealing buffer after 16 h at 4 °C. The gel was run in 10 mM Tris (pH 7.5), 2 mM EDTA, 1 M NaCl, at 80 V, 4 °C for 90 min and stained with GelRed.

Kinetics of Material Formation. Kinetics of the biomaterial formation were measured with diluted mRNA solutions in a cuvette (1 cm path length). First, the mRNA (0.4 μM) was dissolved in 10 mM Tris buffer with 1 M NaCl (pH 7.1), then heated to 80 °C for 5 min and then both RNA hybrids were added in the desired ratios (Table S1, experiments 8-10). The absorption was measured over time at 5 °C. To obtain half-life times, the data points starting at the time point of addition of the hybrids, were fitted to a function for monoexpontential decay using the program QtiPlot. Control experiments with linear strands of the sequences 5'-UUUUUU-3' and 5'-CAUGGU-3' and their complementary strands were performed under the same conditions.

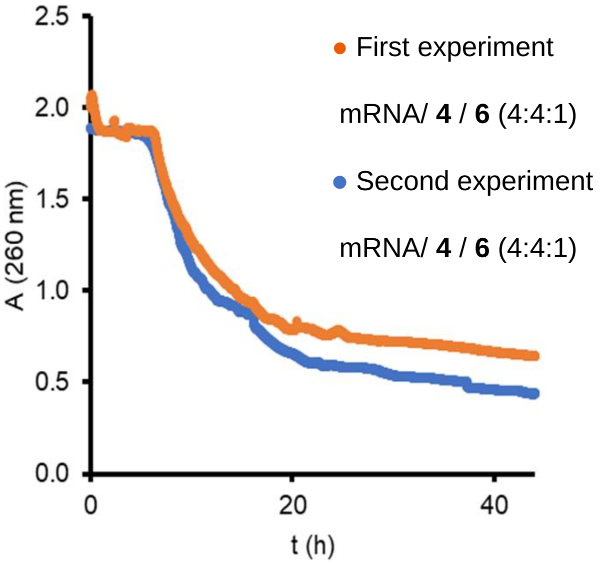
Table S1. Concentrations (μM) of mRNA and RNA hybrids **4** and **6** used in assembly and kinetic assays on biomaterial formation.

experiment No.	c(μM) mRNA	c(μM) hybrid **6**	c(μM) hybrid **4**
1	4	1	-
2	4	4	-
3	4	-	1
4	4	-	4
5	4	1	1
6	4	1	4
7	4	4	4
8	0.4	0.1	0.1
9	0.4	0.1	0.4
10	0.4	0.4	0.4

Reproducibility of Material Formation

Shown below are the results of assays performed in duplicate. The results obtained in the first and the second experiment, performed under the same conditions, are plotted in one graph each.

a)



b)

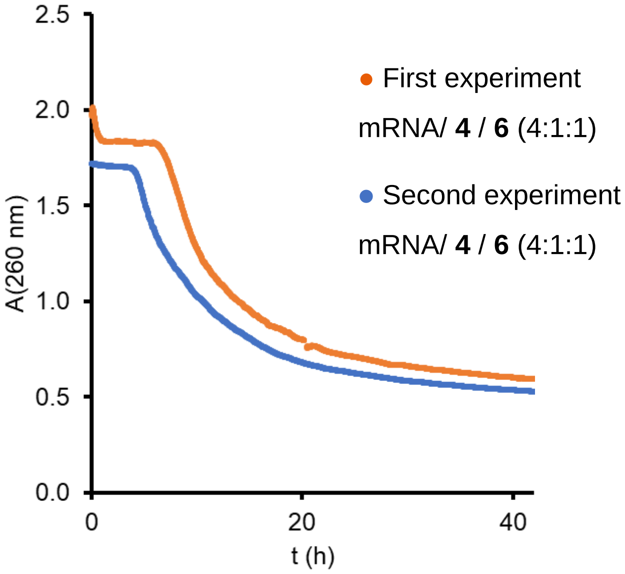


Figure S10. Kinetics of biomaterial formation from mRNA (0.4 μM) and RNA hybrids **4**+**6,** a) at a molar ratio of 4:4:1, and b) at a molar ratio of 4:1:1, as detected by decrease of UV-absorption in two independent experiments each. This shows the reproducibility of the data presented in in Figure 4 of the manuscript. Conditions: 1 M NaCl, 10 mM Tris, pH 7.1, 5 °C. We note that processes like precipitation and crystallization depend on nucleation and other parameters that are difficult to control fully.

Hybridization of Hybrids and Complementary Oligoribonucleotides

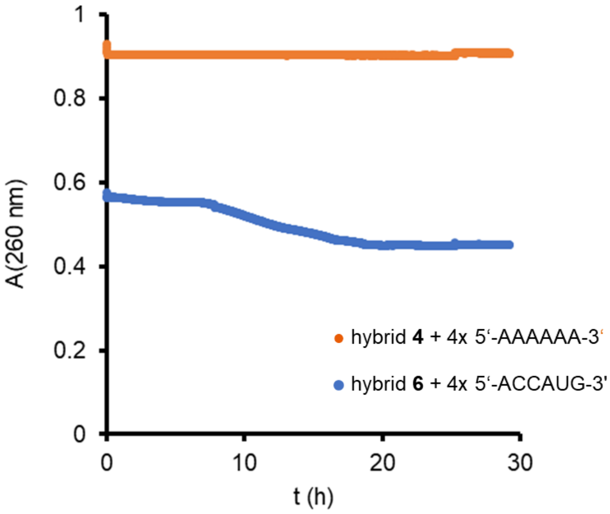


Figure S11. Results of kinetics experiments with RNA hybrids and oligonucleotides complementary to their arms. Either hybrid **4** $(UUUUUU)_4TOA$ (7 µM) or hybrid **6** $(CAUGGU)_4TOA$ (5 µM) and four equivalents of the complementary oligoribonucleotide were mixed in aqueous solution containing 1 M NaCl, 10 mM Tris, pH 7.1, 5 °C and the UV absorbance was measured over time. Neither of the hybrids gave a precipitate, but slow hybridization manifested itself through the hypochromicity expected for duplex formation in the case of **6,** with its more strongly pairing base sequence.

5. Dynamic Light Scattering

Exploratory experiments were performed to determine the size of the particles formed upon assembly of the hybridization networks from mRNA and branched hybrids. A sample of biomaterial, prepared by annealing 4 µM mRNA and 4 µM RNA hybrids **4** and **6** in equimolar concentration from 70 °C to 4 °C over 2 h in 10 mM Tris buffer with 1 M NaCl, pH 7.1, was incubated at 4 °C for 3 d, isolated and then diluted with hybridization buffer and studied by dynamic light scattering, using a Zetasizer Ultra (Malvern Panalytical). Figures S12-S14, below, shows results from these measurements, obtained at different dilutions and angles. On the right-hand side of each figure, the primary data is shown, and the left-hand size shows the size distribution calculated from this data. The legend lists the concentration of mRNA of the dilutions used in the respective experiments.

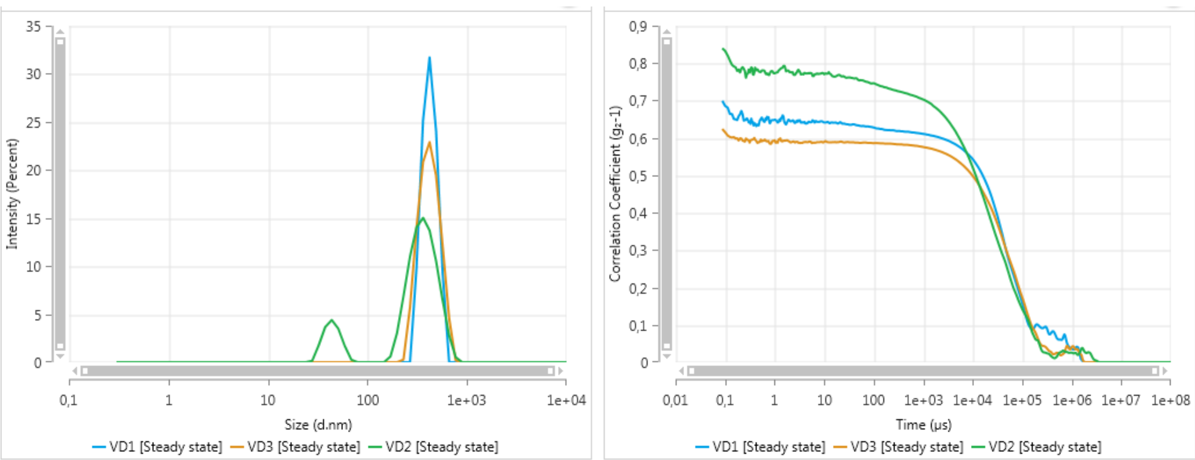


Figure S12. Results of DSL measurements for a sample of biomaterial suspended in hybridization buffer, as obtained at mRNA concentrations of 1 ng/µL (VD1), 5 ng/µL (VD2), and 10 ng/µL (VD3). The particle size distribution is shown on the left, and the plot of the correlation coefficients versus time is shown on the right-hand side.

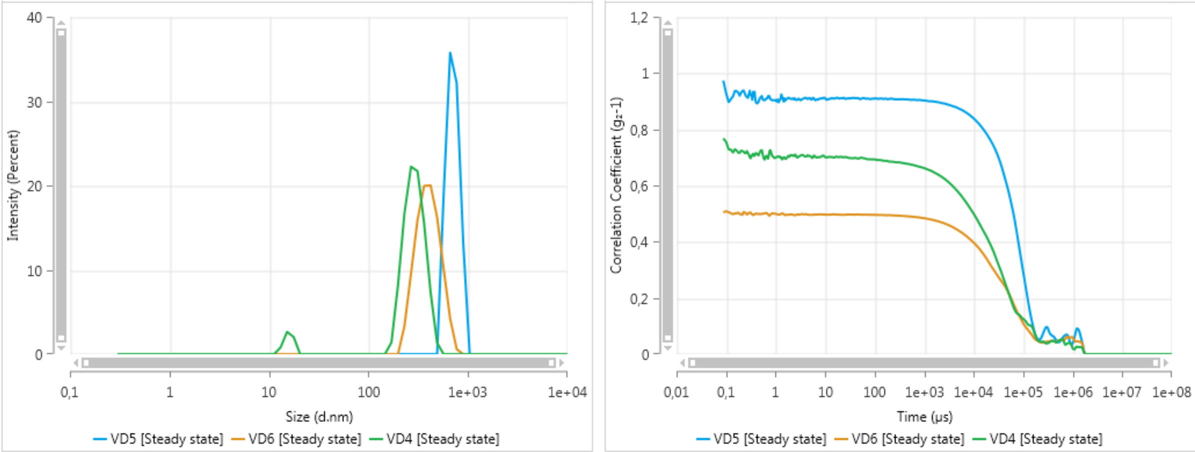


Figure S13. Results of DSL measurements for a sample of biomaterial suspended in hybridization buffer, as obtained at mRNA concentrations of 20 ng/µL (VD4), 50 ng/µL (VD5), and 100 ng/µL (VD6). The particle size distribution is shown on the left, and the plot of the correlation coefficients versus time is shown on the right-hand side.

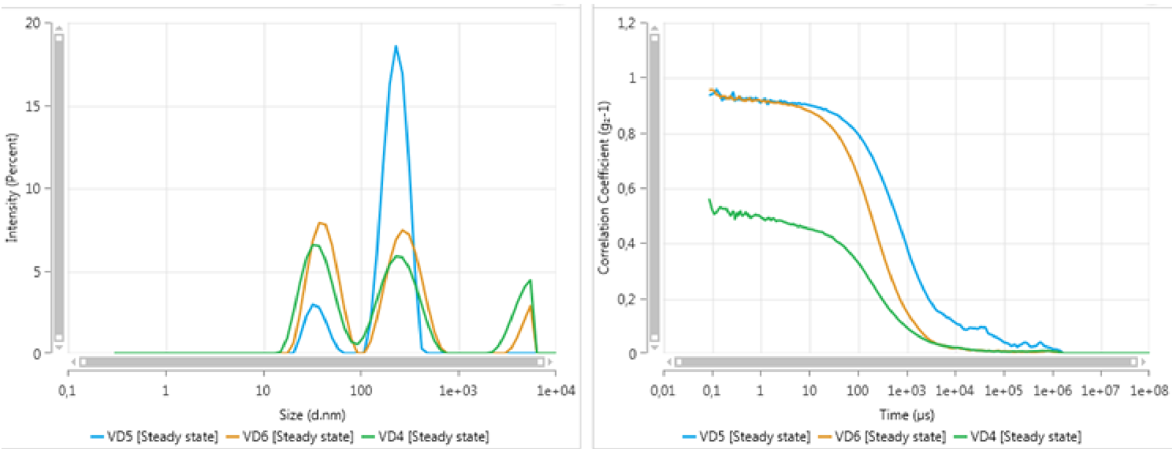


Figure S14 Results of DSL measurements for a sample of biomaterial suspended in hybridization buffer, as obtained at mRNA concentrations of 20 ng/µL (VD4), 50 ng/µL (VD5), and 100 ng/µL (VD6). The particle size distribution is shown on the left, and the plot of the correlation coefficients versus time is shown on the right-hand side. This data set was obtained at a measuring angle of 90°, whereas the data of Figure S13 was measured at an angle of 13°.

References for Supporting Information

S1. M. Fotin-Mleczek, R. Heidenreich (CureVac), WO 2015/149944 A2, **2015**.

S2. A. Schwenger, N. Birchall, C. Richert, *Eur. J. Org. Chem*. **2017**, *39*, 5852-5864